Characterization of Hydroxyapatite from Kupang Shells and its Synthesis with Polycaprolactone for 3D Printing Filament

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***Abstract*— The number of deaths or fatalities due to accidents in Indonesia is increasing. In addition, traffic accidents can cause serious injuries such as damage to the skull. The bone implantation technique currently used is an autograft, but this technique has some limitations. This limitation of autograft can be overwhelmed with synthetic bone implants, one of which uses a mixture of Polycaprolactone (PCL) and Hydroxyapatite (HAp). A combination of HAp and PCL is recommended because the two materials complement each other's weaknesses and can increase elasticity and quality to produce suitable filaments for 3D printing processes. This study used hydroxyapatite from Kupang Shells by calcination and precipitation methods. Then do the test XRF, XRD, FTIR, and SEM to determine the quality. The results showed that HAp synthesized from Kupang Shells had potential characteristics as bone implants. Next, two methods were used to mix PCL with HAp as a 3D printing filament *for bone implants*: the dry and wet methods. After that, it is analyzed with SEM and Mechanical Strength. The results of the SEM test of HAp particles in the wet method have more even distribution and a smoother surface than in the dry method. The impact is visible on the filament's mechanical test, which shows better results in the wet method.**

***Keywords*— 3D printing filament, Hydroxyapatite, Kupang shells, Polycaprolactone**

I. INTRODUCTION

The traffic accident index in Indonesia is very worrying, and this can be seen from the increase in the number of deaths or fatalities due to accidents by 33% in 2018 [1]. Traffic accidents can cause minor to severe injuries. Serious injuries generally occur in the skull. One of the existing bone implantation techniques is an autograft, a bone replacement by placing bone implants from the patient's body. However, this technique has limitations, including a high risk of wound infection, the potential for morbidity, and limited procurement [2]. The limitations of autograft can be overwhelmed through other, safer, and more effective methods by using alternative synthetic bone implants such as polylactic acid polymer (PLA), polyglycolic acid (PGA), and polycaprolactone (PCL) [3] Hydroxyapatite [4], or a mixture of the two (PLA-HAp) [5]. Hydroxyapatite (HAp) is a material that is widely used for implant materials because of its biocompatible and osteoconductive properties [6].

The calcium content of HAp can be produced from biological materials such as coral, clamshells, and eggshells [3]. Pu'ad (2020) using clamshells to synthesize HAp showed excellent and promising results because the calcium carbonate composition was relatively high,around 98% [4]. The quality results of HAp stronglysupport bone-implant research that is currently developing using 3D Printing techniques. This method uses a filamentto form the desired object. The choice of material for the filament is essential because it will affect the quality and mechanical properties of the impression bone implant.

The material for the synthesis of bone implants that are widely used today is a combination of PLA and HAp polymers [5], [7]. However, the use of PLA polymer as bone implant material needs to be reviewed [1]. PLA has low crystallinity, low thermal deformation, and easy brittle. Therefore, it can affect the quality of the resulting bone implants. Besides PLA, another type of polymer is infrequently used for bone implant materials, namely Polycaprolactone (PCL). This type of polymer is a semicrystalline polyester, biodegradable, and more biocompatible. Several PCL devices have been approved by the US Food and Drug Administration because PCL has high flexibility and is very suitable for various fabrication methods [8].

The research being developed currently focuses on two methods in the polymer synthesis process: the dry and wet methods. Research that uses the dry method has been carried out [9]. This study combined polymer and HAp without using a solvent. On the other hand, the wet method used dichloromethane as a solvent for the mixing process [10]. From the two studies, it can be seen that the mixing method used will affect the results of the HAp dispersion into the polymer matrix and the quality of the resulting filament.

From [5] and [9] on the manufacture of bone implants, no study has compared the effectiveness of the dry and the wet method in the manufacture of bone-implant filaments. Therefore, this study aims to synthesize bone-implant composites using PCL-HAp material and then compare the results obtained from the two methods used (dry and wet methods) to mix PCL and HAp. This composite of two materials (PCL-HAp) will be used as a 3D Printing filament material for bone implants.

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II. METHOD

The materials used in this study were mussel shells, CH_3COOH, KH_2PO_4, and Polycaprolactone (PCL). The main equipment used is a furnace, hot plate and stirrer, magnetic stirrer, oven, and extruder machine.

A. *Hydroxyapatite Synthesis*

Clean Kupang Shells were calcined in a furnace at a temperature of 1000ºC for 5 hours. Calcium oxide (CaO) powder was obtained for further X-Ray Fluorescence (XRF) test. Calcined CaO was precipitated by dissolving KH_2PO_4 and stirred at 30 rpm at 37ºC for 30 minutes. The precipitated solution was stored for 24 hours at room temperature and filtered to obtain a white precipitate. The precipitate was washed three times using distilled water. The filtered precipitate was heated at a temperature of 110ºC for 3 hours. Hydroxyapatite was obtained by sintering the dry precipitate at 800ºC for 4 hours. The results of the hydroxyapatite formed were analyzed by X-ray Diffraction (XRD) to identify the crystal size, element, and degree of crystallization of HAp, Fourier Transform Infra-Red (FTIR) to analyze the structure of HAp, and Screening Electron Microscopy (SEM) to analyze the morphology and pore size of HAp.

B. *Synthesis of HAp-PCL Composite*

The manufacture of 3D Printing filaments using the wet method is carried out by dissolving a 20% solution (w/v) in a ratio (w.PCL/v. CH_3COOH) of 10 g of PCL in 50 mL of 100% CH_3COOH and adding HAp powder with a PCL:HAp ratio of 9:1 then stirred for 2 hours. Finally, the mixture was molded and the solvent removed by placing it in the oven for 1 hour at 120ºC.

The dry method synthesis was carried out by heating PCL seeds at 60ºC until melted and adding HAp powder with a PCL:HAp ratio of 9:1 and then stirring until homogeneous. The mixture is molded and cut into small pieces. The PCL-HAp composite was put into the extruder at 70ºC. The filaments formed were analyzed by Screening Electron Microscopy (SEM) and Mechanical Strength (stress, strain, break, and modulus young).

RESULTS AND DISCUSSION

A. *Analysis of the Calcination Results of Kupang Shell*

Calcination is the decomposition and elimination of chemical bonded compounds. For example, magnesium carbonate ($MgCO_3$) decomposes at 540ºC, and $CaCO_3$ is wholly converted to CaO at 1000ºC [11].

The results as shown in Table 1. after calcination at 1000ºC, the most significant component produced is CaO (99.34%). The rest were other components such as MnO, Fe_2O_3, CuO, and SrO. Therefore, the composition of CaO compounds can be more satisfactory because it has a considerable potential to be used as a hydroxyapatite synthesis material in bone implants.

TABLE 1.
XRF RESULTS FROM THE CALCINATION PROCESS OF KUPANG SHELLS *(CORBULA FABA)*

	Component	% Content
Element	Ca	99,15 ± 0,01
	Mn	0,035 ± 0,004
	Fe	0,11 ± 0,01
	Cu	0,055
	Sr	0,635 ± 0,002
Oxide	CaO	99,34
	MnO	0,029 ± 0,003
	Fe_2O_3	0,1
	CuO	0,049
	SrO	0,473 ± 0,011

B. *Hydroxyapatite Characterization of Kupang Shells*

The precipitation method was synthesizing hydroxyapatite (HAp) of Kupang Shells. The synthesis of HAp was carried out by mixing CaO from calcined Kupang Shells with KH_2PO_4 solution using a mole ratio of CaO and KH_2PO_4 of 1.67 (Ca/P).

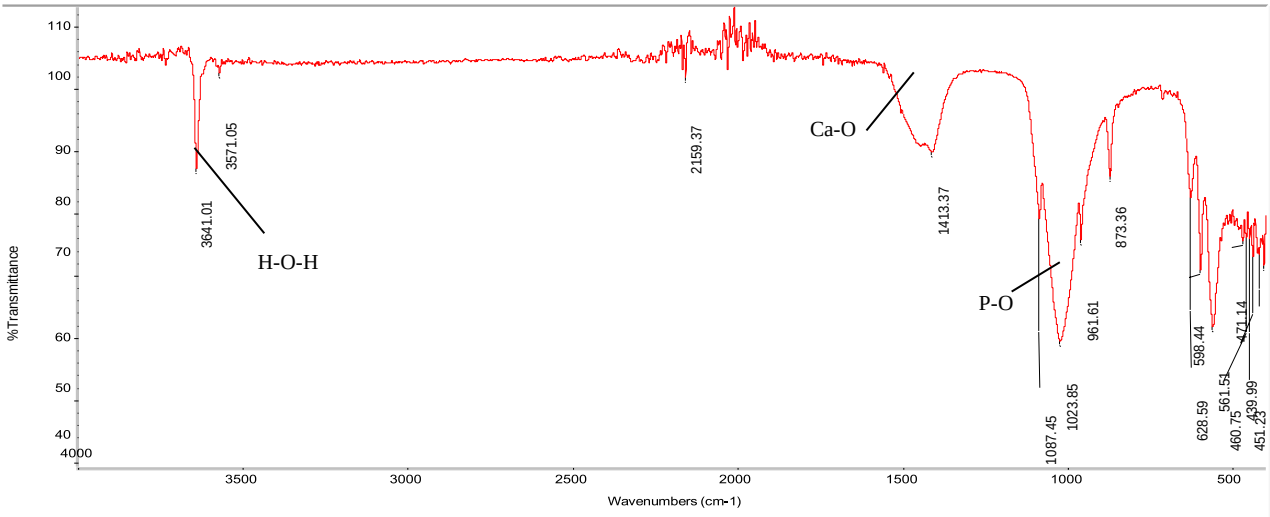


Figure 1. Results of FT-IR HAp analysis from kupang shells

The method is to make a 1.67 M KH_2PO_4 solution by dissolving 114 grams in 500 ml Aquadest. Next, 5 grams of CaO, put it in 250 ml of distilled water, and then drip 32 ml of KH_2PO_4 solution.

1) Characterization of Hydroxyapatite by Functional Group

Transform InfraRed (FTIR) is a tool used to identify types of chemical bonds in calcium phosphate compounds. This characterization identified the HAp functional group. The most typical functional groups in the FTIR spectrum of HAp are PO_4^{3-}, CO_3^{2-}, dan OH^-.

The phosphate group (PO_4^{3-}) is the highest intensity at wavenumbers 1023.85 cm^{-1} and 1087.45 cm^{-1}. According to Pattanayak et al. (2005), the most substantial phosphate group (PO_4^{3-}) bond with stretching vibrations is found at wave intervals of 1000–1150 cm-1 and the medium at wavenumbers 960 cm-1. Infrared spectra, which show the presence of molecular hydrogen bonds, occur at a wavenumber of 3641.01 cm-1, which is indicated by functional group vibrations from H-O-H. Based the results of the existing intensity peaks shows the functional groups that make up the hydroxyapatite compound $[Ca_{10}(PO_4)_6(OH)_2]$. Therefore, it can be concluded that the resulting biomaterial is hydroxyapatite.

The functional group of carbonate compounds (CO_3^{2-}) is indicated by C-O bonds at 1413.37 cm^{-1}. According to [12], the presence of CO_3^{2-} was resulted from the reaction of HAp and CO_2, which may be present in the atmosphere during synthesis. However, the presence of this group cannot be said to be wrong because human bones themselves have CO_3^{2-} about 4 to 6% by weight [10]. Thus, it can be concluded that the HAp is analogous to natural bone apatite.

2) Characterization of Hydroxyapatite based on Crystallinity

X-Ray Diffraction (XRD) serves to identify the crystal size, element, and degree of crystallization of material through the intensity peaks. The XRD analysis data were processed using the Match and showed similarities to the pure HAp XRD data in JCPDS No. 09-432.

TABLE 2.
CRYSTAL SIZE ANALYSIS RESULTS

Peak	Cos θ	λ (Å = 10^{-10} m)	β (FWHM)	L (nm)	Average Size (nm)
1	0,96		0,2005	7,5095	
2	0,95		0,2005	7,5310	
3	0,96		0,2005	7,5170	
4	0,90	1,5406	0,2005	7,9521	7,9684
5	0,97		0,1337	11,1130	
6	0,94		0,2005	7,6812	
7	0,95		0,2339	6,4749	

It is evidenced by the peak characteristic of the primary intensity at the value of 2θ of 31.77; 32.9; 32.17; 49.46; 25.85; 39.79; and 34. The Scherer formula is used to calculate the crystal size and degree of crystallinity where K is a constant for the material of 0.94, the wavelength (λ) used in the XRD tool is 1.5406 nm and is FWHM (Full Width at Half Maximum) of the 2θ scale diffraction line.

The crystal size obtained from calculations using the Scherer formula is presented in Table 2. The average crystal size value is 7.9684 nm. The suitable crystal size for application as bone implants is less than 100 nm because the crystal size in nanometres will strengthen the contact between the implant and bone tissue so that the bone regeneration process can proliferate [13].

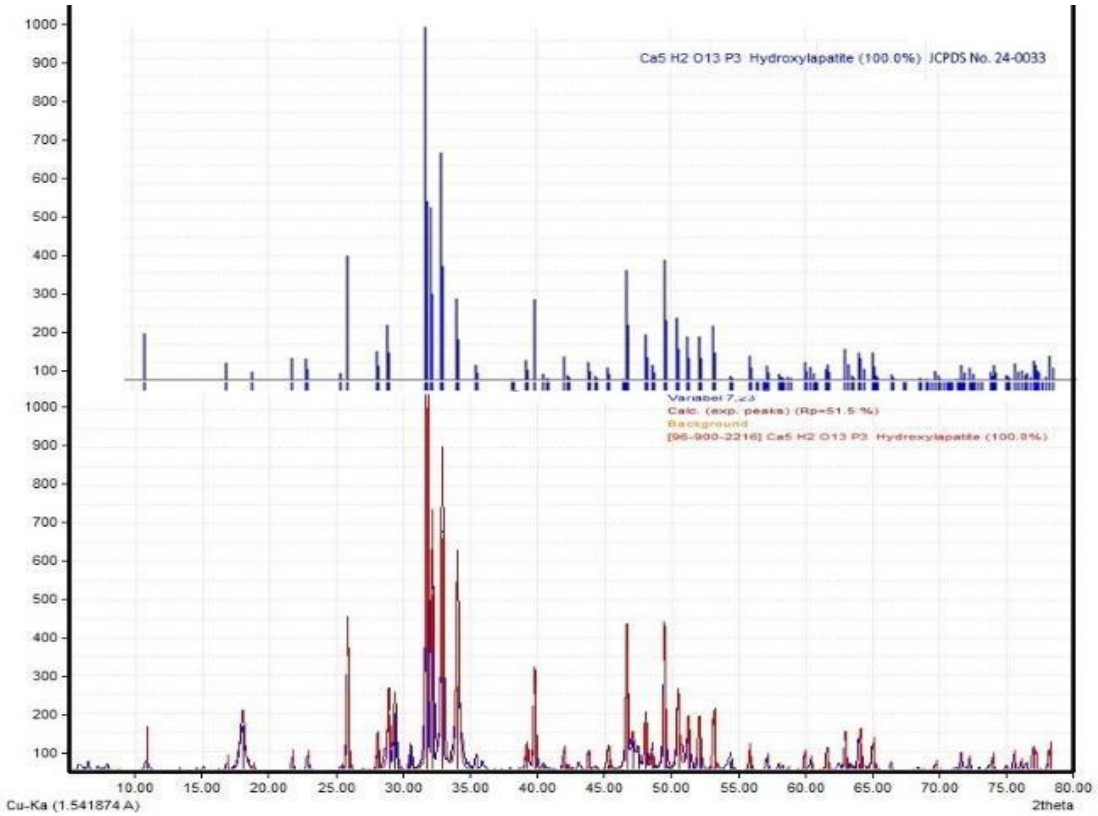
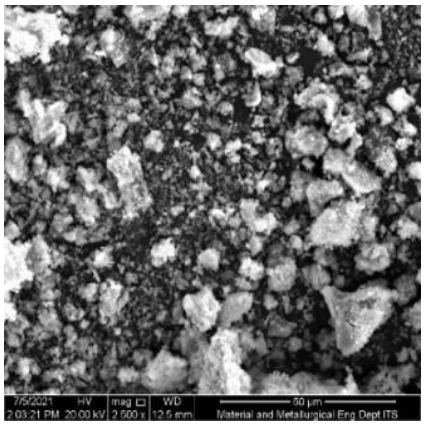


Figure 2. XRD results of the hydroxyapatite synthesis from

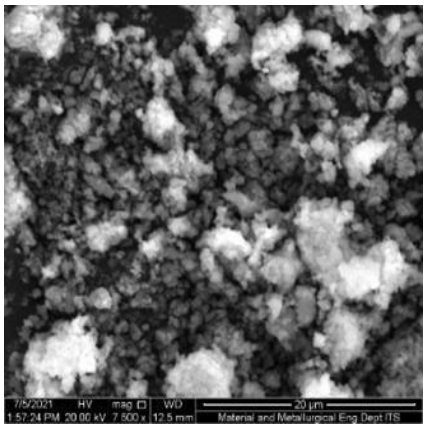
The results of XRD data analysis can also be obtained from the degree of crystallinity of hydroxyapatite from Kupang Shells. The crystallinity value is needed to determine the regularity of the atomic arrangement in a material. Calculate the degree of crystallinity using XRD data processed using Origin Graphing Analysis. Furthermore, the degree of crystallinity is calculated by comparing the crystalline area fraction with the sum of the crystalline area fraction and amorphous area fraction.

According to the calculation, the resulting degree of crystallinity is 87.25% from the XRD data. The formation of a crystal structure causes an increase in crystallinity. In addition, the degree of crystallinity and crystal size is related to the number of impurities and the stoichiometry of the HAp powder. Based on [8], HAp synthesis results generally have a crystallinity of 83.98%. High crystallinity will increase the mechanical strength and high stability and reduce the material's reactivity. Good crystallinity is comparable to good HAp quality [13].

3) Characterization of Hydroxyapatite Based on Morphological



(a)



(b)

Figure 3. Results of SEM analysis from HAp with magnification (a)2500x (b)7500x

Scanning Electron Microscopy (SEM) determines the morphology of a shape material. For example, based on Figure 3. it can be seen that the HAp particles appear to be hexagonal. According to [8], adding a phosphate solution dropwise into a calcium solution causes the addition of Ca^{2+} ions to become slower. Then the HAp crystals grow axially, and the result is in a hexagonal shape.

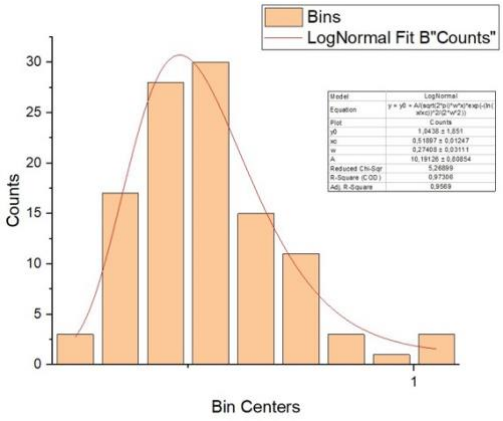


Figure 4. Particle diameter distribution on hap from kupang shell

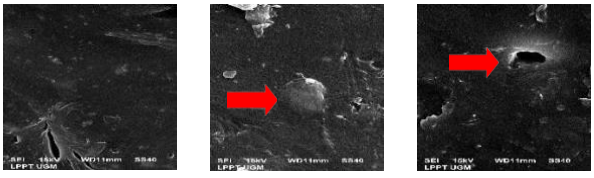
The SEM data were reprocessed using the ImageJ application to obtain data on the distribution of particle diameter sizes from each sample using the sampling method. Finally, the OriginPro application grouped the data to obtain a graph of each sample's particle size distribution, as shown in Figure 4.

The average particle diameter of HAp shells of Kupang Shells is 0.5189 µm. Compared to research conducted by [10], hydroxyapatite synthesized from eggshells has a particle size of 2.21 µm. The smaller HAp particle size will increase the contact surface area of HAp with the surrounding tissue when applied. The size of the surface area is related to the magnitude of the interaction of HAp with bone tissue. Interactions occur between the HAp surface, the bone surface and protein adsorption to form bone growth.

TABLE 3.

FILAMENT RESULTS FROM THE EXTRUSION PROCESS

Method	Diameter (mm)	Filament Result
Dry Method	1.3	
Wet Method	1.28	
Control (Pure PCL)	1.27	



(a) **(b)** **(c)**

Figure 5. SEM results from (a) pure PCL filament, (b) PCL-HAp dry method, (c) PCL-HAp wet method

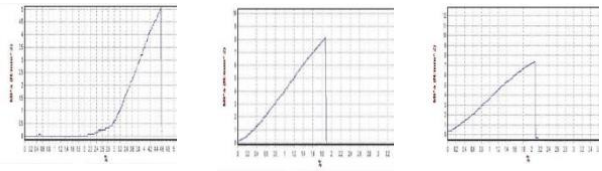
C. 3D Printing Filament Characterization

The results of the synthesis PCL-HAp mixture will become 3D printing filaments for bone implants by extruding. The diameter obtained was uniform, ranging from 1.27 to 1.3 mm (Table 3).

The morphology of the specimen was observed at 5000x magnification. In Figure 5 (a), the surface of the

pure PCL filament looks smooth, while the PCL-HAp filament with the dry and wet methods shows a rough surface. It is due to HAp particles scattered on the surface, as shown in Figures 5 (b) and 5 (c).

The HAp compound was white and the form of granules indicated by arrows, while the synthetic polymer was slightly dark and had a smooth surface. Therefore, it can be seen in Figure 5 (b) that the PCL-HAp filament produced from the dry method has a larger HAp grain size, a rough surface, and an uneven distribution of HAp. Meanwhile, for PCL-HAp filaments produced by the wet method, as shown in Figure 5 (c), the HAp grain size is more uniform and evenly distributed on the PCL surface with a smoother surface than in the dry method.



(a) (b) (c)

Figure 7. Mechanical test of (a) pure PCL filament (b) PCL-HAp filament dry method (c) PCL-HAp wet method

TABLE 4.
MECHANICAL STRENGTH OF 3D PRINTING FILAMENTS

Method	Max Force (N)	Yield Strength (N/mm^2)	Tensile Strength (N/mm^2)	Modulus Young (N/mm^2)	Elongation (%)
Dry method	9.2	4.83	8.15	478.40	1.96
Wet method	8.4	5.39	7.39	410.99	2.16
Control (Pure PCL)	5.7	5.03	5.03	293.71	4.60

Mechanical Strength filament 3D Printing was significantly increased by the addition of hydroxyapatite. Young's modulus in pure PCL increased from 293.71 N/mm^2 to 478.40 N/mm^2. Based on [6], this increase in modulus is sufficient to prevent deformation during bone fabrication and regeneration. In the wet method, the elongation value of 2.16% is better than the dry method required for bone implant applications. There was no significant difference in the Yield Strength value between pure PCL and PCL-HAp with both methods. Yield Strength is influenced by the size and morphology of mineral particles. In the wet method, the Yield Strength value is relatively high, 5.39 N/mm^2. Therefore, the wet method is better than the dry method in making 3D printing filaments for bone implants.

IV. CONCLUSION

The Kupang Shell is potential as a raw material for synthesizing hydroxyapatite because it has a high CaO content of 99.34%. The characterization of hydroxyapatite synthesized from Kupang Shells showed good quality and quantity in terms of functional groups, crystallinity, and morphology. The average value of the hydroxyapatite particle size is 7.968 nm, and the crystallinity value is 87.27 % which follows the specifications for making bone implants. The crystal structure of HAp was a hexagonal shape. The results of the characterization of 3D Printing filaments produced from the dry method have less uniform HAp sizes and a rougher particle surface when compared to PCL-HAp filaments produced by the wet method. This also affects the mechanical test results of the wet method filament, which is better than the dry method

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